Original Article

KRAS and NRAS Testing in Metastatic Colorectal Cancer in Central Iran (Tehran): A Review on Literature of the Middle East

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Abstract

Context: The incidence of colorectal cancer (CRC) in the past three decades in Iran has made it as a major public health burden**. Aims:** The aim of this study is to report the prevalence of KRAS and NRAS mutations in Iran and the correlation between KRAS mutation status with clinicopathological factors and survival. **Materials and Methods:** In a cross-sectional study, 144 patients were entered into the study based on the criteria. Age, sex, tumor site, grade, metastasis location, familial history, KRAS/NRAS status, and survival were checked for all patients, and the patients were followed for 1 year. DNA was extracted with FFPE QIAGEN kit and then polymerase chain reaction for amplification of gene segments of KRAS and NRAS genes. **Results:** The mean age at diagnosis was 52.9 years (range: 27–72 years) that 39.6% patients had age <50 years and 54.2% were men. KRAS mutation was significantly more in the patients with age ≥50 compared with KRAS wild type. Furthermore, the 6-month overall survival rate in KRAS mutation patients was significantly more than KRAS wild-type patients. Liver metastasis (72.9%) had the highest prevalence of metastasis in the patients, and Grade II with 64.6% had the most prevalence. **Conclusions:** The metastatic CRC was more prevalent in men than women, and the mean age varied around 50–60 years. The results showed that the present study had the highest prevalence of KRAS mutation in the Middle East and Pakistan with the lowest prevalence in CRC patients.

Keywords: *Colorectal cancer, Iran, KRAS, NRAS*

Introduction

Colorectal cancer (CRC) is one of the most common cancers and is the second leading cause of cancer death in men and women in the United States and also is third common cancer in women and fifth in Iranian men.[1] The increasing incidence of CRC in the past three decades in Iran has made it as a major public health burden.[2] In CRC patients, metastases are the main cause of cancer-related mortality.[3] The most common sites of metastasis from colon cancer are the regional lymph nodes, the liver, the lung, and the peritoneum.[3,4] Distant metastatic disease is present in approximately 25% of patients at initial diagnosis, and half of CRC patients will develop metastatic disease.[5] Most patients with metastatic CRC (mCRC) have an incurable disease.[3] The treatment of mCRC is one of the biggest successes in recent decades.[6] Targeted cancer therapy is becoming a powerful strategy for the treatment of patients selected on the basis of their molecular characteristics. This is particularly true for patients with mCRC.[2] RAS mutations are useful markers for predicting responses to anti-epidermal growth factor receptor monoclonal antibodies in mCRC.[2,7] KRAS mutation varies between 20% and 50% in the most countries in the world,[8] but NRAS mutations are rare and occur in 3% and 5% of CRC.[9] The frequency of NRAS mutations and their relationship to clinical, pathologic, and molecular features remains uncertain.[9]

The aim of this study is another report from the prevalence of KRAS and NRAS mutations in Iran and the correlation between KRAS mutation status with clinicopathological factors and survival.

Materials and Methods

Patients

The cross-sectional study was done in CRC patients in Rasool Akram Hospital, Tehran, in 1 year (April 2015 to April 2016) that 144 patients were entered into the study based on inclusion criteria.

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Inclusion criteria

Patients having mCRC and history of treatment with chemotherapy regimens were included in the study. Age, sex, tumor site, grade, metastasis location, familial history, KRAS/NRAS status, and survival were checked for all patients. The patients were followed up for 1 year. The overall survival was defined as the length of time from either the date of diagnosis or the start of treatment for cancer until death for any cause or the date of the last follow-up.

Extraction and amplification of KRAS/NRAS

Mutations and oncogenes of KRAS and NRAS of codons 12 and 13 were checked on fresh frozen and formalin-fixed paraffin-embedded (FFPE) tissues in Partolab Laboratory, Tehran, Iran. After 4–5 cutting with 2–5 μm thickness, DNA was extracted with FFPE QIAGEN kit, and then, polymerase chain reaction (PCR) for amplification of gene segments was done with an initial denaturation at 95°C for 11 min, denaturation at 95°C for 30 s, fusion at 55°C for 30 s, elongation at 72°C for 30 s, and end elongation at 72°C for 5 min. After electrophoresis of PCR products, a band in the situation of 120 bp was seen that is indicating amplification of gene segment during PCR. To ensure amplified fragment length to confirm the target gene, evaluation was performed by Gene Runner program, and the incision with enzyme was confirmed by MapViewer program. After that, using RFLP technic and suitable enzymes (Bgl1), the status of mutation and wild type was determined. The results again were checked with high-resolution melting analysis. KRAS and NRAS genes were analyzed and sequenced (pyrosequencing) by allele specific.

Statistical analysis

The analysis was done with IBM SPSS software version 22 (IBM Corp., Armonk, NY, USA) that *t*-test was used for the comparison of means between groups and Chi-square test for other variables. The overall survival was plotted and analyzed by Kaplan–Meier.

Results

The mean age ± standard deviation at diagnosis was 52.9 ± 12.7 years (range: 27–72 years) that 39.6% patients had age <50 years and 54.2% were males [Table 1]. Sigmoid (33.4%), ascending colon (31.2%), rectum (20.8%), descending colon (8.4%), and transverse colon (6.2%) were the highest tumor site in the patients. Grades I (well differentiated), II (moderate differentiated), and III (poorly differentiated) were 16.7%, 64.6%, and 18.7%, respectively. In all patients, liver metastasis (72.9%) had the highest prevalence, followed by nonregional lymph node and lung (each 10.4%) and other metastases (6.3%). Of 144 patients, 15 patients had a familial history of cancer, 72 (52.1%) had KRAS mutation, and 6 (4.2%) had NRAS mutation. In addition, during 1-year follow-up, 51 (35.4%) patients died for any cause.

Table 1: Characteristics of all patients (*n*=144)

Variables	*n* (%)
Age (years)	
Mean±SD	52.9±12.7
Range	27-72
<50	57 (39.6)
Sex	
Male	78 (54.2)
Female	66 (45.8)
Tumor site	
Ascending colon	45 (31.2)
Transverse colon	9 (6.2)
Descending colon	12 (8.4)
Sigmoid	48 (33.4)
Rectum	30 (20.8)
Grade	
I	24 (16.7)
II	93 (64.6)
III	27 (18.7)
Metastasis location	
Liver	105 (72.9)
Nonregional lymph node	15 (10.4)
Lung	15 (10.4)
Other	9 (6.3)
Familial history	
Yes	15 (10.4)
No	129 (89.6)
KRAS status	
Mutation	75 (52.1)
Wild type	69 (47.9)
NRAS status	
Mutation	6 (4.2)
Wild type	138 (95.8)
One-year survival	
Alive	93 (64.6)
Deceased	51 (35.4)

SD – Standard deviation; KRAS – Kirsten ras; NRAS – Neuroblastoma ras

Table 2 compares the characteristics of the patients based on KRAS status. There was just a significant difference between KRAS mutation patients and KRAS wild-type patients ($P < 0.001$) that KRAS mutation was more in the patients with age ≥50 but KRAS wild type in the patients with age <50 years.

The comparison of 1-year overall survival based on KRAS status has been shown in Figure 1. The 6-month overall survival rate in KRAS mutation patients (84%) was more than KRAS wild-type patients (98.6%) ($P = 0.002$). Furthermore, the 1-year overall survival rate was 68% in KRAS mutation patients versus 60.9% in KRAS wild-type patients ($P = 0.371$).

Discussion

This study showed that the prevalence of KRAS and NRAS

Table 2: The comparison of characteristics of the patients based on KRAS status

Variables	KRAS mutation (*n*=75) (%)	KRAS wild type (*n*=69) (%)	*P*
Age (years)			
Mean±SD	54.64±13.11	51.09±12.04	0.093
<50/≥50	18 (24)/57 (76)	39 (56.5)/30 (43.5)	<0.001
Sex			0.645
Male	42 (56)	36 (52.1)	
Female	33 (44)	33 (47.9)	
Tumor site			0.574
Ascending colon	24 (32)	21 (30.4)	
Transverse colon	6 (8)	3 (4.3)	
Descending colon	6 (8)	6 (8.7)	
Sigmoid	21 (28)	27 (39.2)	
Rectum	18 (24)	12 (17.4)	
Grade			0.914
I	12 (16)	12 (17.4)	
II	48 (64)	45 (65.2)	
III	15 (20)	12 (17.4)	
Metastasis location			0.436
Liver	57 (76)	48 (69.5)	
Nonregional lymph node	6 (8)	9 (13.1)	
Lung	9 (12)	6 (8.7)	
Other	3 (4)	6 (8.7)	
Familial history			0.322
Yes	6 (8)	9 (13.1)	
No	69 (92)	60 (86.9)	

SD – Standard deviation; KRAS – Kirsten ras

mutations in mCRC patients in an Iranian population was 52.1% and 4.2%, respectively. Furthermore, KRAS mutation had significantly more prevalence in lower ages compared with KRAS wild type.

One study[10] in Western Iran on 83 mCRC patients (mean age: 57.7 years, range: 18–80 years, and 61.4% males) and another study on 33 mCRC patients[11] (mean age: 51.5 years, range: 22–76, and 79% males) in this area reported that the prevalence of KRAS mutation was 33.3% and 44.6%, respectively. Furthermore, there were no significant differences between patients with KRAS mutation and wild type in 5-(69% vs. 64%)[10] and 2-year (63% vs. 73%) overall survivals.[11] Two studies[10,11] showed that the prevalence of clinicopathological factors in patients with KRAS mutation and wild type was similar. Two studies in Tehran (Central Iran)[1,12] evaluated mCRC patients for KRAS status that the first study selected 1000 cases (57.3% males) with the prevalence of 33.6% for KRAS mutation, and the second study selected 182 CRC patients with the prevalence of 37.4% for KRAS mutation. Koochak *et al*.[13] indicate that KRAS mutations occurred at a statistically higher frequency in older patients (>50) than in younger patients (≤50) (P = 0.0001). It is also worth mentioning that KRAS mutation tended to occur

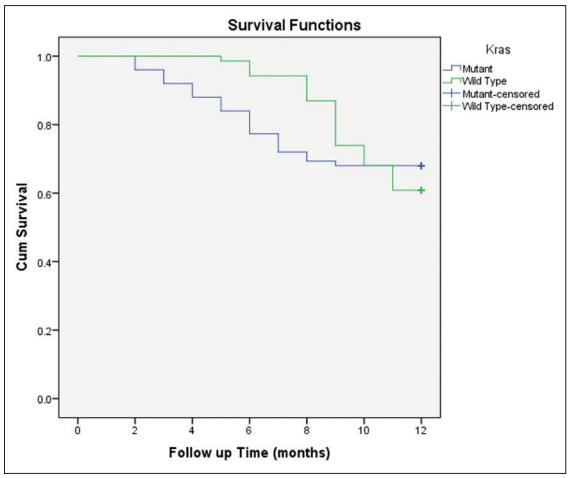

Figure 1: The comparison of 1-year overall survival based on KRAS status

at a more frequency in male cases than in female cases. KRAS mutations occur at a higher rate in pT3 than others (P = 0.0001). The studies in other areas of Iran reported 32% KRAS mutation in 100 cases (mean age: 59 years and 55% males) in Southern Iran,[14] 32.2% in 211 cases in Northern Iran,[13] and 28% in 50 cases (mean age: 60.8 years), and NRAS mutation was just in one case in codon 146 (2%) in Southern Iran.[15] The prevalence of NRAS mutation in Iranian CRC patients has been reported from 0%[6,10,11] to 4.2% (the present study). One review study[7] showed that the prevalence of KRAS mutation in Iran was between 30% and 50% (few studies reported 12.5%–37.4%) that this prevalence was similar to studies from other countries (20%–50%).

A total of 83 mCRC patients evaluated in Saudi Arabia[16] that 42.2% had KRAS mutation, and 51% and 23% of the tumors were from the left hemi-colon and rectum, respectively, 83% were moderately differentiated, and 86% were invasive adenocarcinoma. Another study on 300 CRC patients from Saudi Arabia[17] reported 42% KRAS mutation that mutations were associated with advanced stage of CRC and shorter recurrence-free survival and overall survival.

The studies reported in Turkey[18-20] checked 50, 172, and 53 mCRC patients. The prevalence of KRAS mutation was 30%, 44%, and 49.05%, respectively. KRAS mutation frequency was significantly higher in tumors located in the ascending colon,[18] and there was no difference in progression-free survival and overall survival between KRAS mutation and KRAS wild-type patients.

One hundred and fifty CRC patients (64% male) in Pakistan were assessed for KRAS status that 13% had the mutation, and this mutation seemed to be significantly associated with female patients.[21] One research in Egypt[22] analyzed KRAS status on 26 mCRC patients with immunohistochemistry. The results showed that 42.3% patients were KRAS mutation, and no significant

correlation was found between clinicopathological parameters and KRAS staining results.[22] In Oman,[23] 79 CRC patients were checked that 48.1% had mutation and no relation was noticed with wild-type or mutant KRAS with recurrence-free survival and overall survival. In Iraq,[24] fifty CRC patients (mean age: 55.4 years and 54% males) enrolled that 48% had KRAS mutation, and there were no significant associations of age, gender, tumor location or histology, grading, staging, or lymphovascular invasion with KRAS mutation status**.** In the present study, there was no significant difference between clinicopathological factors with KRAS mutation status, except for age that KRAS mutation frequency was higher in age ≥50 years. Furthermore, there was no significant difference between KRAS mutation status for 1-year overall survival, but 6-month overall survival was significantly shorter in KRAS mutation compared with KRAS wild type.

Conclusions

The mCRC was more prevalent in men than women, and the mean age varied around 50–60 years. The results showed that the present study had the highest prevalence of KRAS mutation in the Middle East and Pakistan with the lowest prevalence in CRC patients. The studies reported that survival of the patients was similar between KRAS mutation and wild type, but in the present study, 6-month survival had a significant difference. In the future, studies can be considered to the survivals of shorter than 1 year in mCRC patients. In addition to, in more studies, there was no significant difference between KRAS mutation status with clinicopathological factors but can be considered to the role of age, stage, and tumor location in the future studies.

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Conflicts of interest

There are no conflicts of interest.

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